ORIGINAL ARTICLE

PCSK9 Levels Are Increased in Type 2 Diabetes Mellitus with, and without Statin Therapy in Thai Subjects

Chutima Rattanawan, PhD[1,4], Burabha Pussadhamma, MD[2,3], Nutjaree Jeenduang, PhD[1]

[1] School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, Thailand; [2] Department of Internal Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [3] Queen Sirikit Heart Center of the Northeast, Khon Kaen University, Khon Kaen, Thailand; [4] Hematology and Transfusion Science Research Center (HTSRC), Walailak University, Nakhon Si Thammarat, Thailand

Background: Plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) levels were found associated with not only lipid profiles, but also glucose homeostasis. Nevertheless, the relationship between PCSK9 levels, and diabetes was inconsistent in various studies.

Objective: To investigate the plasma PCSK9 levels among normoglycemia, pre-diabetes, and T2DM patients with, and without statin therapy in Thais.

Materials and Methods: Five hundred fifty-three subjects including 213 normoglycemia, 176 pre-diabetes, 46 T2DM without statin therapy, 40 T2DM with simvastatin therapy at 20 mg/day, and 78 T2DM with simvastatin therapy at 40 mg/day were recruited. Anthropometric data, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood sugar (FBS), and PCSK9 levels were measured.

Results: PCSK9 levels were significantly higher in T2DM without statin therapy compared with normoglycemic subjects. In addition, T2DM with simvastatin therapy (40 mg/day) had significantly higher PCSK9 levels than T2DM without statin therapy, and T2DM with simvastatin therapy (20 mg/day). Serum PCSK9 levels were positively correlated with several metabolic parameters including age, body mass index (BMI), systolic blood pressure (SBP), TC, TG, and FBS ($p<0.05$) in the present study subjects.

Conclusion: PCSK9 levels were modulated by glycemic status, and statin therapy in Thai subjects. Elevation of plasma PCSK9 levels in T2DM with, and without statin therapy may increase the risk for dyslipidemia, and cardiovascular disease among T2DM patients.

Keywords: PCSK9, Pre-diabetes, T2DM, FBS, Statin

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Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia, results from insulin resistant. Dyslipidemia is one of the major risk factors for cardiovascular disease (CVD) in diabetes mellitus[1]. Diabetic dyslipidemia is characterized by elevated triglyceride (TG), TG-rich lipoproteins (TRLs), small dense low-density lipoproteins (sdLDLs), and reduced high density lipoprotein (HDL) levels. Diabetic dyslipidemia increases the incidence of atherosclerosis, CVD, and mortality in diabetic patients[2].

Correspondence to:

Jeenduang N.

School of Allied Health Sciences, Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat 80160, Thailand.

Phone: +66-75-672678, **Fax**: +66-75-672601

Email: nutjaree.je@wu.ac.th

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is the ninth member of the serine protease family that is mostly synthesized in liver[3]. PCSK9 plays an important role in cholesterol homeostasis[3,4]. It binds low-density lipoprotein receptors (LDLR) on the hepatocyte membrane, and promotes the internalization, and rapid degradation of LDLR in lysosomes; thereby, resulting in hypercholesterolemia[3,4]. PCSK9 levels are modulated by factors such as age, gender, body mass index (BMI), dietary intake, hormone, exercise, medicinal plants, and lipid-lowering therapy[5]. Currently, PCSK9 inhibitors (PCSK9i) such as alirocumab and evolocumab have been approved by the U.S. Food and Drug Administration (FDA) and are effective in the reduction of LDL-C levels, and adverse cardiovascular events in patients with and without diabetes mellitus[6].

Previous studies showed that PCSK9 levels were positively correlated with TC, and low-density lipoprotein cholesterol (LDL-C)[5]. Nevertheless, studies demonstrated that PCSK9 levels were also

positively correlated with fasting glucose, and insulin[7-12]. Plasma PCSK9 levels were significantly higher in diabetes than patients without diabetes in the Dallas Heart Study[7], the United Arab Emirates National Diabetes Study[8], Tunisia[9], Spain[10], Chinese Han[11], and Korean[12] populations. In contrast, plasma PCSK9 levels were not significantly different between diabetes, and the control group in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study[13], and Northern Finland population[14].

In Thailand, T2DM and pre-diabetes were found in approximately 9.9% and 15.4%, respectively[15]. Plasma PCSK9 levels in diabetes, and pre-diabetes in Thai population have not been studied. Therefore, in the present study, the authors investigated the PCSK9 levels in pre-diabetes, T2DM patients with and without statin therapy, and compared with normoglycemic subjects.

Materials and Methods

Subjects and sample collection

Five hundred fifty-three subjects including 213 normoglycemia, 176 pre-diabetes, 46 T2DM without statin therapy, 40 T2DM with simvastatin therapy (20 mg/day), and 78 T2DM with simvastatin therapy (40 mg/day), matched by gender, were recruited in the present study. Normoglycemia, pre-diabetes, and T2DM without statin therapy were recruited from the general population in Nakhon Si Thammarat Province. T2DM with statin therapy were recruited from the outpatient department (OPD), Thasala Hospital, Nakhon Si Thammarat. Normoglycemic was defined as fasting blood sugar (FBS) of less than 100 mg/dL, pre-diabetes was defined as FBS 100 to 125 mg/dL, and T2DM was defined as FBS of 126 mg/dL or more or intake of anti-diabetic medication[16]. BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured. Exclusion criteria for study subjects were the presence of chronic diseases, and the use of medicines, such as hormone replacement therapy (HRT), oral contraceptives, and drug abuse. The medication in T2DM patients was recorded by using the questionnaire, and medical record. Written informed consents were obtained from all subjects before being included in the present study. The study protocol was approved by the Ethics Committee of Walailak University (protocol No. WUEC-19-016-01, WUEC-16-057-01), and Thasala Hospital.

Laboratory analysis

Blood samples were collected from the subjects after 12-hour fasting. The serum, and plasma were separated by centrifugation at 3,000 rpm for 10 minutes. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and TG were measured using standard enzymatic method. LDL-C was calculated using the Friedewald formula. FBS was measured using the glucose oxidase method. All tests were performed by using the Konelab analyzer (KONELAB 20, Tokyo, Japan). For PCSK9 measurement, serum was collected, aliquoted after overnight fasting and stored at –80°C. PCSK9 levels in serum were measured using a commercially available quantitative sandwich ELISA assay following the manufacturer instructions (Biolegend, San Diego, CA).

Statistical analysis

All data were analyzed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation (SD). Differences between the two groups were tested using the student t-test for parametric factors, and the Mann-Whitney U test for non-parametric factors. For multiple comparisons of means among groups, the one-way ANOVA, ANCOVA, and Kruskal-Wallis test were performed. Spearman correlation was used to analyze the correlation between PCSK9 levels, and biochemical parameters. A p-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics

The basic characteristics of study subjects are summarized in Table 1. There was no difference in the proportion of male and female among the group studies. BMI, SBP, TC, TG, and FBS levels were significantly higher in pre-diabetes and T2DM without statin therapy than in the normoglycemic group ($p<0.05$). BMI and FBS were significantly higher in T2DM without statin therapy than pre-diabetes ($p<0.05$). Furthermore, age, SBP, DBP, TC, and TG were significantly higher in T2DM with simvastatin (40 or 20 mg/day) than T2DM without statin therapy ($p<0.05$). Plasma PCSK9 levels were significantly higher in T2DM without statin therapy than normoglycemic subjects (Figure 1). T2DM with simvastatin therapy 40 mg/day showed significantly higher PCSK9 levels than T2DM with 20 mg/day simvastatin therapy, and T2DM without statin therapy (Figure 2).

Table 1. General characteristics of study population

Variables	No statin therapy; mean±SD			Simvastatin therapy; mean±SD		p-value# normoglycemia vs. pre-diabetes vs. T2DM	p-value# T2DM vs. T2DM with simvastatin (20 mg/day) vs. simvastatin (40 mg/day)
	Normoglycemia (n= 213)	Pre-diabetes (n= 176)	T2DM (n= 46)	T2DM (20 mg/day) (n= 40)	T2DM with (40 mg/day) (n=78)		
Male/female	69/144	57/119	14/32	8/32	21/57	0.965	0.537
Age (years)	54.72±10.17	56.74±12.52	55.83±12.07	61.90±10.26[d]	61.13±11.03[e]	0.153	0.010
BMI (kg/m^2)	23.66±4.19	24.26±4.33	26.87±4.93[b,c]	28.35±5.37	26.29±4.19	<0.001	0.169
SBP (mmHg)	129.51±17.91	135.51±20.72[a]	136.65±20.78[b]	130.90±8.08	129.09±9.58[e]	0.007	0.029
DBP (mmHg)	79.71±11.03	79.55±12.52	83.61±13.48	73.40±7.77[d]	72.00±8.44[e]	0.158	<0.001
TC (mg/dl)	168.34±80.55	211.56±46.04[a]	214.43±48.34[b]	208.20±53.64	244.94±76.58[e,f]	<0.001	0.013
TG (mg/dl)	111.92±63.43	131.10±56.14[a]	138.98±64.67[b]	172.43±71.48[d]	172.13±79.13[e]	<0.001	0.036
HDL-C (mg/dl)	60.17±18.14	60.83±18.20	60.39±14.73	63.63±18.83	62.12±19.51	0.780	0.839
LDL-C (mg/dl)	123.72±33.28	124.49±42.38	126.30±42.49	110.09±39.29	148.39±66.52[f]	0.894	0.007
FBS (mg/dl)	90.49±5.93	107.89±6.33[a]	160.41±41.91[b,c]	145.48±33.45	151.59±50.47	<0.001	0.101
PCSK9 (ng/ml)	79.17±29.24	81.86±24.22	88.07±26.78[b]	91.03±15.01	112.42±38.44[e*,f*]	0.036	<0.001*

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglyceride; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; FBS=fasting blood sugar; PCSK9=proprotein convertase subtilisin/kexin 9

[a] Normoglycemia vs. pre-diabetes, p<0.05; [b] Normoglycemia vs. T2DM, p<0.05; [c] Pre-diabetes vs. T2DM, p<0.05; [d] T2DM vs. T2DM with simvastatin 20 mg/day, p<0.05; [e] T2DM vs. T2DM with simvastatin 40 mg/day, p<0.05; [f] T2DM with simvastatin 20 mg/day vs. T2DM with simvastatin 40 mg/day, p<0.05; [a-f] p-value analyzed by Student t-test, or Mann-Whitney U test

* p-value adjusted by age analyzed by ANCOVA, # p-value analyzed by ANOVA, or Kruskal-Wallis test

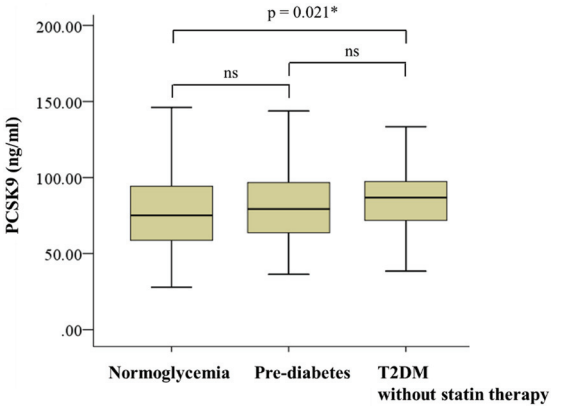

Figure 1. Comparison of plasma PCSK9 levels among normoglycemia, pre-diabetes, T2DM without statin therapy.

* Normoglycemia vs. T2DM without statin therapy, p<0.05, p-value analyzed by Mann-Whitney U test; ns=not significant

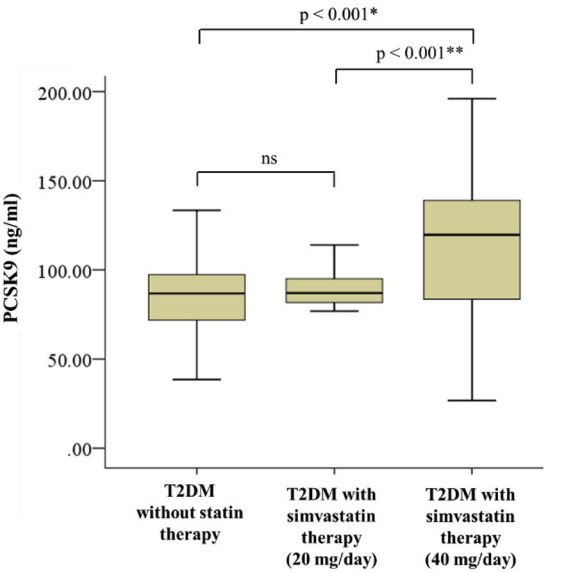

Figure 2. Comparison of plasma PCSK9 levels among T2DM with, and without statin therapy.

* T2DM without statin therapy vs. T2DM with simvastatin therapy (40 mg/day), p <0.001; ** T2DM with simvastatin therapy (20 mg/day) vs. T2DM with simvastatin therapy (40 mg/day), p <0.001, p-value analyzed by Mann-Whitney U test; ns, not significant

Serum PCSK9 levels correlate with cholesterol and other metabolic parameters

The Spearman correlation analysis between PCSK9 levels and biochemical parameters are shown in Table 2. PCSK9 levels were positively correlated with age, SBP, TC, TG, and FBS in all subjects, and normoglycemic subjects. In addition, PCSK9 levels had weakly positively correlated with BMI, and negatively correlated with DBP in all subjects. Moreover, PCSK9 levels were positively correlated with age in pre-diabetes, and T2DM without statin therapy, and positively correlated with TC in T2DM with simvastatin therapy (20 mg/day), but negatively correlated with FBS in T2DM without statin therapy.

Table 2. Correlation analyses between plasma PCSK9 levels and biochemical parameters

Dependent variables	All subjects; r	No statin therapy; r			Simvastatin therapy; r	
		Normoglycemia	Pre-diabetes	T2DM	T2DM (simvastatin 20 mg/day)	T2DM with (simvastatin 40 mg/day)
Age (years)	0.249***	0.220**	0.212**	0.507***	–0.141	0.119
BMI (kg/m^2)	0.091*	0.023	–0.058	0.060	–0.059	0.017
SBP (mmHg)	0.111*	0.176*	0.074	0.049	–0.179	0.145
DBP (mmHg)	–0.143**	0.022	–0.0134	–0.162	0.142	–0.092
TC (mg/dL)	0.221***	0.371***	0.076	0.007	0.355*	–0.088
TG (mg/dL)	0.213***	0.299***	0.052	–0.054	0.266	–0.075
HDL-C (mg/dL)	0.056	0.039	0.062	–0.017	0.308	0.173
LDL-C (mg/dL)	–0.016	–0.118	0.043	0.030	0.240	–0.134
FBS (mg/dL)	0.262***	0.198**	0.0132	–0.300*	0.166	–0.069

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglyceride; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; FBS=fasting blood sugar

* p<0.05, ** p<0.01, *** p<0.001; p-value analyzed by Spearman correlation

Discussion

The present study found that the higher PCSK9 levels was observed in T2DM without statin therapy but not in pre-diabetes compared with normoglycemia. Moreover, the higher PCSK9 levels was also observed in T2DM with simvastatin therapy (40 mg/day) compared with T2DM with simvastatin therapy (20 mg/day), and T2DM without statin therapy.

The results were consistent with several studies[8-12,17,18]. Plasma PCSK9 levels were significantly higher in T2DM than non-diabetic subjects or control group in the United Arab Emirates National Diabetes Study[8], Tunisia[9], Spain[10], and Chinese Han[11] populations, as well as, in youth in USA[17]. Moreover, PCSK9 levels were also significantly higher in subjects with T1DM[18]. The elevation of PCSK9 levels in T2DM could be induced by hyperinsulinemic condition[19]. The study in rat hepatoma cells and primary rat hepatocytes showed that insulin increased PCSK9 expression, and increased LDLR degradation. Whereas, the study in insulin receptor knockout mice, hepatic PCSK9 mRNA, and plasma PCSK9 protein levels were reduced by 55% to 75%[19]. Even though, the exact mechanisms of insulin on regulation of PCSK9 was not completely clarified, the previous studies demonstrated that insulin could induce the PCSK9 expression via sterol regulatory element-binding protein-1c (SREBP-1c), whereas it inhibited the PCSK9 expression by suppression of hepatocyte nuclear factor-1 alpha (HNF-1α). In normal human physiology, the effect of insulin on the induction, and inhibition of PCSK9 expression was balanced[19,20].

In contrast, PCSK9 levels were found significantly higher in subjects with impaired glucose regulation (IGR) compared with normal glucose tolerance (NGT) in Chinese Han[11], and Korea[12] populations. Moreover, a few studies showed that plasma PCSK9 levels were not significantly different between diabetes, and control group in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study[13], and the Northern Finland population[14]. The discrepancy results may result from the different ethnicity, sample size, and underlying diseases of the study subjects.

In the present study, T2DM treated with simvastatin 40 mg/day had significantly higher PCSK9 levels (+27.65%) than T2DM without statin therapy. Whereas, a slightly increased PCSK9 levels (+3.36%) in T2DM treated with simvastatin 20 mg/day compared with T2DM without statin therapy was observed. Moreover, T2DM treated with simvastatin 40 mg/day showed significantly higher PCSK9 levels (+23.50%) than T2DM treated with simvastatin 20 mg/day. These suggested that PCSK9 levels may increase according to dose of simvastatin. A previous study supported that PCSK9 levels increased according to dose, and duration of statin therapy[21]. Moreover, the present study results were consistent with the previous studies in which PCSK9 levels were increased in statin-treated patients in Finland[14], in France[22], in USA[23], and in the Dallas Heart Study[12]. The study in HepG2 cells[24,25], and in mice[26] demonstrated that statin activated the transcription factor, sterol regulatory element-binding protein 2 (SREBP-2), and then increased in LDLR and PCSK9 mRNA levels[27]. The higher levels of

PCSK9 can promote the degradation of LDLR, and then may lead to the lower lipid-lowering efficacy of statin therapy[23].

The present study, PCSK9 levels were positively correlated with SBP, TC, and TG in all subjects, and normoglycemic subjects. PCSK9 were also positively correlated with age in all subjects, normoglycemia, pre-diabetes, and T2DM without statin therapy. Several studies demonstrated that PCSK9 levels were significantly positively correlated with age, and serum lipids, such as TC, TG, and LDL-C in the Dallas Heart Study[7], a large population of 2,719 Han Chinese[28], in 1,739 French Canadian youths in the Quebec Child and Adolescent Health and Social Survey[29], in 254 healthy Canadians[30], and in 52 Caucasian participants in the Netherlands[31].

In the present study, there was no association between PCSK9 and LDL-C levels in all subjects, pre-diabetes, and T2DM without statin therapy. Similarly, the subjects in Canada[32], adult females in the United Arab Emirates[33], and healthy subjects and T2DM patients in Tunisia[9] did not show the correlation between PCSK9 and LDL-C levels. The lack of association between PCSK9 levels, and LDL-C levels may be related to the forms of PCSK9 in detection. In the present study, the authors used sandwich ELISA to measure both mature, and furin-cleaved PCSK9 forms. A previous study supported that the total forms of PCSK9 were not surrogated by active PCSK9[34]. In addition, the furin-cleaved form, a biologically inactive form, was also not significantly correlated with oxidized LDL-C (oxLDL-C)[35]. Moreover, the diurnal changes, and the fasting that affected the wide fluctuations of PCSK9 concentrations[36,37] may be the additional factors to cause the loss of relationship between PCSK9 and LDL-C levels.

Moreover, the PCSK9 levels were not correlated with LDL-C levels in T2DM with statin therapy in the present study. The results were similar to the previous studies in which the correlation between PCSK9, and LDL-C levels was blunted in the patients treated with statin[38]. In addition, another study showed a negative correlation between PCSK9 percentage change and LDL-C percentage change in patients treated with rosuvastatin[39]. This may be due to statin treatment that can reduce LDL-C, and increase PCSK9 expression simultaneously.

Finally, the authors found that PCSK9 levels were positively correlated with FBS in all subjects, normoglycemic subjects, but negatively correlated with FBS in T2DM without statin therapy. The correlation between PCSK9 levels, and FBS seemed to vary among various studies[7,28,29,40,41]. The positive correlation between PCSK9 and FBS levels was also observed in Chinese adults[28]. However, PCSK9 levels were positively correlated with insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), but they were not correlated with FBS[29,40]. Moreover, the positive correlation between PCSK9 and insulin, and HOMA-IR were found in Dutch Caucasians[41], and in the Dallas Heart Study[7]. This is suggesting that insulin and HOMA-IR may be the important predictors for presenting the association between PCSK9 and glucose homeostasis.

The present study has limitations. The sample size was small, particularly in the T2DM with, and without statin therapy. The duration, and compliance of statin therapy were not recorded. In addition, HbA1C, insulin, and HOMA-IR were not measured.

Conclusion

PCSK9 levels were modulated by the glycemic status and statin therapy in Thai subjects. Elevation of plasma PCSK9 levels in T2DM with, and without statin therapy may increase the risk for dyslipidemia and CVD among T2DM patients.

What is already known on this topic?

PCSK9 levels are found associated with lipid profiles and glucose homeostasis. Nevertheless, the relationship between PCSK9 levels and diabetes is inconsistent in various studies.

What this study adds?

PCSK9 levels are significantly higher in T2DM without statin therapy compared with normoglycemic subjects. Whereas, PCSK9 levels are not significantly different between pre-diabetes, and normoglycemic subjects. This indicated that the increased PCSK9 levels may increase the risk for dyslipidemia and CVD among T2DM patients.

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Conflicts of interest

The authors state that there is no conflict of interest.

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